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Binding of ethidium bromide causes dissociation of the nucleosome core particle

(chromatin/intercalation/DNA)

CYNTHIA T. MCMURRAY AND K. E. VAN HOLDE*

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331

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ABSTRACT **The binding of ethidium bromide to chicken erythrocyte core particles results in a step-wise dissociation of the structure that involves the initial release of one copy each of histones H2A and H2B. Quantitation of the dissociated DNA reveals that a critical amount of drug is required for the dissociation. Above the critical value, the dissociation is time dependent, reversible, and independent of DNA concentration.**

Ethidium bromide binds by intercalation between the base pairs (bp) of DNA (1, 2). The binding properties of intercalating molecules are of particular interest since compounds of this class include mutagens (3), chemotherapeutic agents (4), and carcinogens (5). While extensive investigation has been undertaken to elucidate the base-pair specificity and sequence requirements of many intercalating dyes to free DNA (6, 7), little is known about how chromatin structure modifies their binding, or how the binding affects chromatin structure. This is particularly surprising since the *in vivo* target of many mutagens and carcinogens appears to be the nucleic acid folded into chromatin. At the level of the 30-nm fiber, ethidium bromide promotes the release of histone H1 (8) and thus has the potential to disrupt higher-order structure. At the level of the 10-nm fiber, the binding of ethidium bromide derivatives (9) has indicated that the linker segment is preferentially bound relative to the nucleosome. This binding preference, however, conflicts with a report (10) that the affinity to the nucleosomal form of the DNA is 10-fold greater relative to free DNA. In general, little agreement has been reached concerning dye-binding affinity, cooperativity, and structural consequences of intercalation (10, 11) to the core particle.

Ethidium bromide, its derivatives, and other intercalating agents have been used to investigate the motional dynamics of the core particle (12, 13), to analyze nucleosome phasing in active genes (14), and to identify torsional stress in the prokaryotic and eukaryotic genomes (15, 16). To evaluate such experiments, a clear understanding of the effects of intercalation on nucleosome structure is required.

In this initial study, we report that a major structural consequence of binding ethidium bromide to the core particle is a time-dependent dissociation.

MATERIALS AND METHODS

Preparation of Chicken Erythrocyte Core Particles. The chicken erythrocyte core particles were prepared by a modification of the Lutter (17) procedure. Long monomers (145–200 bp), from the second micrococcal nuclease digest, were purified and trimmed to 145 ± 3 bp by a third enzymatic digestion.

Electrophoresis. *Native core particle gels.* Native core particle gels were prepared as described (18).

Protein gels. The protein components were analyzed by standard $NaDodSO_4$/polyacrylamide gel electrophoresis described (19) and stained with Coomassie blue.

Two-dimensional gel electrophoresis. Core particle–ethidium bromide complexes were analyzed in the first dimension on native core particle gels (18). Each band was cut from the native gel and soaked for 5 min in O'Farrell's buffer (20). The one-dimensional bands were analyzed on a $NaDodSO_4$/protein gel, composed of a 6% stacking gel and 15% separating gel that was stained with Coomassie blue as described by Laemmli (19).

Sucrose Gradient Centrifugation. Ethidium complexes with the core particle and free DNA were analyzed on 5–20% linear sucrose gradients buffered with 10 mM Tris·HCl, pH 8.0/0.1 mM EDTA prepared as described (18). Samples were centrifuged immediately after mixing. The sample concentration was 4–8 × 10^{-4} bp (A_{260}, 5–10).

Equilibrium Dialysis. Equilibrium dialysis experiments were performed in a three-chambered Plexiglass apparatus; each block contained eight separate cells. The central chamber contained the buffer plus ethidium bromide; thus, every sample was measured in duplicate. The buffer used for all experiments was 10 mM Tris·HCl/0.1 mM EDTA, pH 8.0. The core particle concentration was 3.8 × 10^{-5} M bp. Ethidium bromide was added to the central chamber and allowed to equilibrate for 2–4 days at 30°C while gently shaking. The concentration of free ethidium bromide was determined directly by measuring the absorbance of the central chamber at 480 nm and dividing by the extinction coefficient of free ethidium bromide, 5750 $M^{-1}\cdot cm^{-1}$ (2).

Sedimentation Velocity, Circular Dichroism, and Thermal Denaturation. All procedures were described (18, 21).

5′-End-Labeling. Core particle stocks at 9.5 × 10^{-4} M bp (A_{200}, 12.5) were labeled at their 5′-DNA termini with ^{32}P as described (17).

RESULTS

Integrity of the Core Particle. PAGE (Fig. 1*A*) of the native core particle (lane 1) and DNA extracted from the core particles (lane 3) showed an extremely homogeneous preparation. Plots of log molecular weight versus relative mobility (data not shown) of the marker DNA fragments were used to determine the size of the core particle DNA. The plot indicated that the core length ranged from 142 to 148 bp and is, therefore, taken as 145 ± 3 bp. Our preparation displayed no trace of higher molecular weight oligomer, free DNA, or significant levels of subnucleosomal material. The sedimentation coefficient of the intact cores in 10 mM Tris·HCl, pH 8.0/0.1 mM EDTA was 10.8 ± 0.2S at 20°C, in excellent

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Abbreviation: bp, base pair(s).

*To whom reprint requests should be addressed.

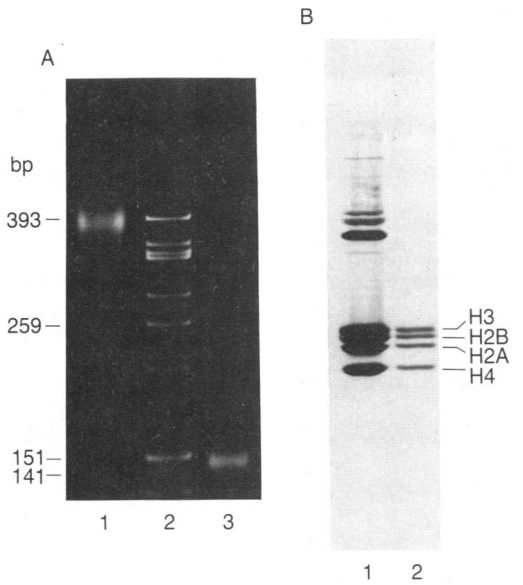

FIG. 1. (*A*) Gel electrophoretic analysis of the nucleosome core particles (lane 1) and DNA isolated from the core particles (lane 3). Polyacrylamide gels were 1.0 mm thick containing 3.5% polyacrylamide (acrylamide/bisacrylamide; 20:1; wt/wt). Lane 2 contains DNA molecular size markers from *Cfo* I-digested pBR322. Lane 1 shows 30 ng of the native core particle in 10% (vol/vol) glycerol/10 mM Tris·HCl, pH 8.0/0.1 mM EDTA. Lane 3 contains 50 ng of the DNA isolated from the core particles in standard Laemmli sample buffer. Gel electrophoresis was performed at 4°C at 10 V/cm for ≈50 min. The gels were stained for 20 min with ethidium bromide at 1 μg/ml. (*B*) Laemmli $NaDodSO_4$/PAGE of histones. Gels were 0.8 mm thick containing 15% polyacrylamide (acrylamide/bisacrylamide; 30:0.8; wt/wt) in the separating gel and 6% acrylamide in the stacking gel. Lane 2 contains 1.0 μg of total protein from our core particles. Lane 1 represented 3.12 μg of chicken-erythrocyte whole chromatin histones. Samples (A_{260}, 1.0) in 10 mM Tris·HCl/0.1 mM EDTA, pH 8.0, were boiled in Laemmli $NaDodSO_4$ sample buffer (19) for 1 min, cooled on ice, and loaded onto an Idea Scientific minigel apparatus. Gel electrophoresis was performed at 4°C at 15 V/cm for 1 hr. The gels were stained overnight with 0.25% Coomassie blue and destained in methanol/acetic acid according to the procedure of Laemmli (19).

agreement with previous studies (21, 22), thus confirming the integrity of our preparation.

Analysis of protein on standard Laemmli gels (Fig. 1*B*), stained with Coomassie blue, demonstrated that our preparation (lane 2) lacked any significant amounts of nonhistone proteins, any trace of histones H1 or H5, or any degradation products of the inner core histones. The circular dichroism spectrum had the following characteristic features of native nucleosomes: a small negative peak at ≈295 nm, a double peak exhibiting a maximum ellipticity of 1400 ± 200 deg·cm^2·mol^{-1} at 282.4 nm, and a zero-crossover point at ≈270 nm. Inflection points of the thermal melting profiles in 10 mM Tris·HCl/0.1 mM EDTA, pH 8.0, were in excellent agreement with previous results (21).

The Binding of Ethidium Bromide to the Nucleosome Core Particle Results in Dissociation. Fig. 2 shows the results of electrophoretic analysis of the effect of ethidium bromide binding on core particle. Lanes 1–12 represent increasing input ratios of ethidium bromide to core particle bp, equilibrated for 15 hr after mixing. In the absence of drug (lane 1), we observed only a single band (band 1) that migrated near the 404-bp marker DNA band (*Hpa* II-digested pBR322) as expected for the intact core particle (see Fig. 1*A*, lane 1). Increasing the input ethidium bromide to core particle bp molar ratio of 0.10–0.20 (lanes 3 and 4) resulted in the appearance of a second band (band 2) that migrated between the 404-bp and 309-bp marker bands. The presence of band

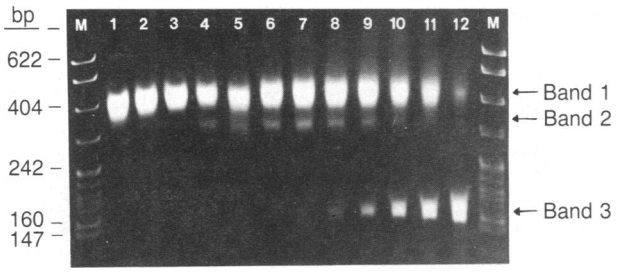

FIG. 2. Results of the polyacrylamide gel electrophoretic analysis of the core particle–ethidium bromide complexes over a range of input ratios. Complexes were analyzed 15 hr after mixing. In lanes 1–12, the R values (mol of ethidium bromide per mol of bp) were 0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.45, 0.5, 0.75, 1.0, 1.5, and 2.0, respectively. Lanes marked M indicate *Hpa* II-digested pBR322 DNA molecular size markers. Bands 1, 2, and 3 represent the three products formed from the binding of ethidium bromide to the core particle (see text). Each lane contains 250 ng of DNA. Core particle DNA concentration was 3.8×10^{-5} bp, and the buffer used for all samples was 10 mM Tris·HCl, pH 8.0/0.1 mM EDTA. Gel electrophoresis was performed as in Fig. 1*A*.

2 was observed at ratios above 0.10 and persisted even at dye to bp ratios as high as ≈1.0. At input ratios above 0.2, we identified a third form (band 3) that comigrated with deproteinized nucleosome-sized DNA standards (lane 3; Fig. 1*A*). Band 3 was tentatively assigned as free DNA. Between input ratios of 0.1–2.0 (lanes 3–12), the intensity of band 3 increased at the expense of both bands 1 and 2 until, at a ratio of 2.0, it was the major component. Thus, the gel analysis indicated that two structurally different forms resulted from binding of ethidium bromide to the core particle. One of those forms appeared to be free DNA.

The intensities of both band 2 and band 3 increased when the samples were equilibrated at 30°C for longer periods of time. Fig. 3 displays the results of the electrophoretic analysis of a core particle–ethidium bromide complex (R = 0.5, where R = the core particle/ethidium bromide molar ratio) separated at two times after mixing. Lane A represents the complex immediately after mixing (0 hr). The mixing plus the loading time was ≈1 min. The laser densitometer trace of lane A revealed that most of the intensity for the complex was seen in band 1, with a small amount (≈7%) of intensity in band 3. After 36 hr, however, the intensity distribution had changed significantly. Laser densitometer tracing of lane B revealed the presence of band 2, which was absent immediately after mixing. Additionally, the amount of free DNA had increased to 25%. It is clear that band 2 and band 3 arise at the expense of the intact core structure (band 1), since the appearance of these bands was concomitant with a decrease in the intensity of band 1. At all ratios measured between 0.05 and 1.0, increased levels of band 2 and band 3 were seen relative to the zero time point. We concluded that the binding of ethidium bromide to the core particle resulted in a time-dependent dissociation of the native structure to free DNA, proceeding through at least one intermediate form.

Conclusive identification of bands 1, 2, and 3 is shown by two-dimensional PAGE analyses in Fig. 4. In the first dimension, a core particle–ethidium bromide complex (R_I, 0.3) was separated on a 3.5% native core particle gel. We observed bands 1, 2, and 3 in exact agreement with Fig. 2. Each band was cut from the gel and analyzed in the second dimension on an $NaDodSO_4$/15% polyacrylamide standard protein gel. Densitometer tracings of the protein bands are included in the figure. As expected from its mobility on the gel, band 1 displayed the full complement of all four histone proteins in equimolar quantities. Comparision with Fig. 1 verified that this band represented the native core particle. Similarly, we identified band 3 as free core particle DNA,

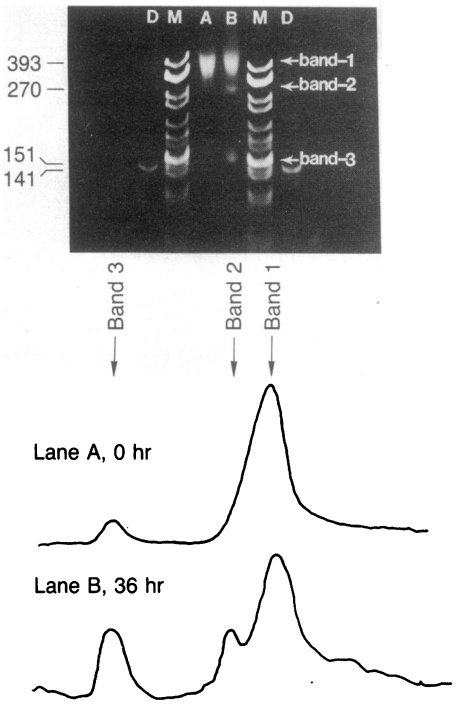

FIG. 3. Time dependence of the step-wise dissociation of the core particle by ethidium bromide at 30°C. (*Upper*) At an input ethidium bromide to core particle bp molar ratio of 0.5, the core particle–ethidium bromide complex was analyzed by 3.5% polyacrylamide gel electrophoresis. Lane A (0 time) represents the core particle complex analyzed immediately after mixing (the mixing and loading time was ≈1 min). Lane B (36 hr) represents the core particle–ethidium bromide complex equilibrated for 36 hr after mixing. M and D refer to Cfo-digested pBR322 DNA molecular size markers and deproteinized 145 ± 3 bp chicken erythrocyte DNA standards, respectively. (*Lower*) Laser densitometer tracings of lanes A and B are shown.

since its migration in the first dimension was identical to that of purified nucleosomal DNA (Fig. 3, lane D); and we observed no protein component (dimension 2) associated with this band. Finally, band 2 was identified as a DNA–histone complex that contained only six of the eight histones. This form shall be referred to as the hexamer. In the second-dimension analysis of band 2, all four histones were present; however, histones H2A and H2B were clearly depleted. Laser densitometer tracing and integration of the protein peak areas showed the ratio of H2A + H2B area relative to H3 + H4 area was equal to 0.5, indicating that only one H2A–H2B dimer was present in band 2. Thus, prior to complete dissociation, one copy each of histones H2A and H2B is lost.

We conducted extensive controls on the gel system that we used, varying the sample load, concentration, ionic strength, glycerol content, and voltage. In all cases we found that both the presence and degree of dissociation were not an artifact of the electrophoretic method. However, to further insure that dissociation was not gel dependent, we analyzed the core particle–ethidium bromide complexes by sucrose gradient centrifugation. Sucrose gradient analysis (Fig. 5) showed the presence of only three distinct peaks that were analyzed for both protein and DNA content. The results of the DNA gel electrophoresis corresponding to the central fraction of each peak (peaks numbered 1–4) can be seen at the bottom of Fig. 5. We determined that peak 1, peak 2, and peaks 3 plus 4, observed in the gradients, corresponded to the native octamer, the hexamer, and free DNA, respectively. The peak labeled "eth" in Fig. 5 was determined to be free ethidium.

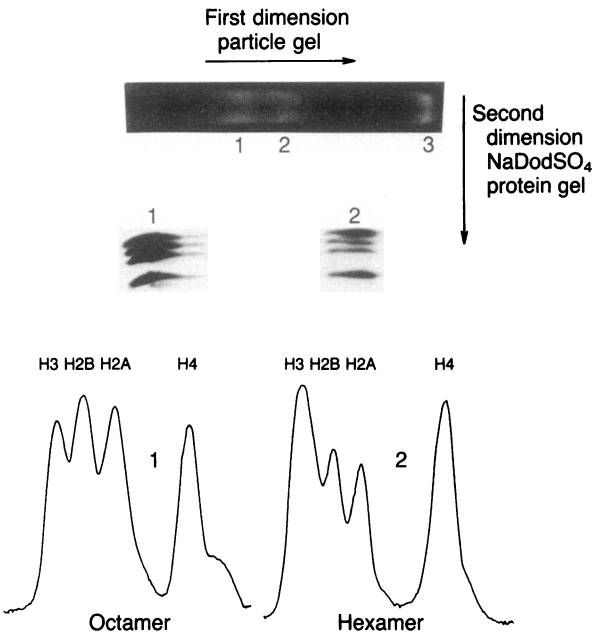

FIG. 4. Two-dimensional gel electrophoretic analysis of the core particle–ethidium bromide complex at an input ratio of 0.3. The core particle complex was analyzed in the first dimension on a 1 mm, 3.5% native polyacrylamide gel (acrylamide/bisacrylamide; 20:1; wt/wt). Gel conditions are identical as described in Fig. 2. Band 1 is the intact core particle; band 2 is the hexamer; band 3 is free DNA. Each band was cut from the first dimension gel and sealed with agarose onto the top of a second dimension 15% $NaDodSO_4$/polyacrylamide Laemmli (19) protein gel and later stained with Coomassie blue. The resulting protein bands were scanned with a laser densitometer, and the peak areas were integrated by a cutting and weighing procedure.

This peak was a red band on the top of the sucrose gradients and contained no detectable DNA or protein.

Ethidium bromide complexes with the core particles were prone to aggregation at high DNA concentrations ($>7.8 \times 10^{-5}$ M bp), especially at high dye to bp ratios and higher ionic strength. We emphasize that both the hexamer and free DNA, bands 2 and 3, occur in the total absence of aggregation. However, quantification was limited to specific conditions. For the sucrose gradients, where the DNA concentration was very high ($4.0–8.0 \times 10^{-4}$ M bp), aggregation was observed at input ratios above 0.3. No aggregation was observed below this ratio. For the equilibrium dialysis and gel electrophoresis experiments, where the DNA concentration was usually low ($\approx 4 \times 10^{-5}$ M bp), no aggregation was observed in solution at any input ratio of ethidium bromide. However, when the complexes were analyzed by gel electrophoresis, aggregation could be detected at the top of the lane above an R of 0.5 due to increasing amounts of free histone. Therefore, we have limited our quantitative analysis only to regions where no aggregation was observed on the gel, i.e., to DNA concentrations of $3.8–7.8 \times 10^{-5}$ M (A_{260}, 0.5–1.0) and drug input ratios ranging from 0 to 0.5.

Quantification of Dissociation. On an ethidium bromide-stained gel, the fluorescence staining of free DNA is more intense than that of core particles. As a consequence, quantitative assessment of the amount of DNA produced at a particular ratio of dye to particle could never be calculated from the fractional intensities of the three bands. We, therefore, quantified the amount of free DNA over a range of input ratios between 0 and 0.5 by two different electrophoretic methods. In method 1, a series of six accurately determined DNA standards were electrophoresed through each gel. All standards, which migrated as tight bands, were scanned with a laser scanning densitometer, and the area was determined by cutting and weighing the respective peaks; this

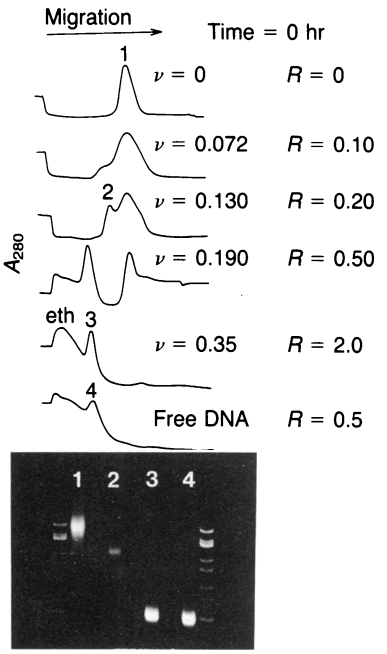

FIG. 5. Results of the linear 5–20% (wt/vol) sucrose gradient centrifugation of the ethidium bromide complexes with the nucleosome core particle and free DNA. Time = 0 hr indicates that the samples were loaded immediately after mixing, and the arrow indicates the direction of migration. The ν = mol of ethidium bromide bound per mol of bp; R = mol of ethidium bromide added per mol of bp. Peaks 1–4 correspond to the gel lanes at the bottom of the figure. Peak 1 is the intact core particle; peak 2 is the hexamer; peak 3 is ethidium-induced dissociated free DNA; and peak 4 is a free DNA–ethidium complex. The peak marked "eth" is free ethidium bromide found at the top of the gradient. Sucrose gradients and core particle stocks were buffered with 10 mM Tris·HCl, pH 8.0/0.1 mM EDTA. The concentration of the core particle samples was 7.8×10^{-4} M bp (A_{260}, 10). Gradients were centrifuged for 24–30 hr at 25,000 rpm in a Beckman SW40 rotor at 4°C.

yielded a plot of peak area versus ng of DNA. In the remaining lanes, quantitative volumes of ethidium bromide–core particle complexes were loaded onto the gel. Since the DNA is well separated from the intact core particle and the hexamer, we could easily scan the free DNA band that appeared at each input ratio of ethidium bromide and determine its amount by comparison with the calibration curve. The fraction of core particles that had dissociated was calculated by the ratio of free DNA to the known amount of core particle DNA loaded onto the gel. All measurements were made within the linear response ranges of both the film and the instrument systems used. The samples used in these experiments were taken from the sample cell in a three-chambered equilibrium dialysis apparatus. Thus, for every input ratio, we could directly determine the fraction of dye actually bound. Method 2 involved the use of end-labeled core particles. 5′-End-labeled core particles were mixed with unlabeled core particles at all input ratios of ethidium bromide. The ethidium bromide–core particle complexes were separated on native gels and autoradiographed, and the films were scanned with the laser densitometer. The results of a typical experiment are listed in Table 1. Only ratios below 0.5 were quantified. We note that the total intensity is relatively constant in this range of input ratios and that the percentage population of the hexamer ranges from 7 to 17%. Since the end-labeling procedure produced a small amount of free DNA, the actual amount of ethidium-induced free DNA was calculated by subtracting the amount of free DNA in the R = 0 lane from the total percentage of free DNA calculated at each ratio.

Table 1. Calculated relative populations of octamer, hexamer, and free DNA for ethidium bromide–^{32}P-labeled core particle complexes at 30°C

Input ratio*	% octamer†	% hexamer‡	% DNA	Total area§
0	95	ND	5	80.8
0.05	94	ND	6	84.3
0.10	94	+	6	87.5
0.20	83	10	7	80.3
0.25	72	17	11	88.8
0.30	73	17	10	84.1
0.35	69	10	20	91.0
0.40	70	7	23	84.6

*Input ratio is mol of ethidium bromide per mol of DNA bp.
†% octamer is (peak area/total area) × 100.
‡ND, not detectable. +, the presence of a hexamer band. Hexamer was detected as a slight shoulder on the octamer peak but was not resolved enough to quantify.
§Total area is total area of scanned lane from autoradiogram of ^{32}P-labeled core particles at indicated ratios—arbitrary units.

The results of the percentage of free DNA calculated from both methods are shown in Fig. 6. The amount of dissociation appeared to be dependent on the bound ratio of dye in a smooth, but nonlinear manner. Extrapolation of the curve to zero dissociation indicated that dissociation did not occur at the lowest levels of ethidium bromide binding; but rather that a critical amount of the compound was required to be bound before dissociation began. This value, called ν_c, was determined to be 0.06. Since the core particle length is 145 bp of DNA, a ν_c = 0.06 indicates that ≈9 ethidium molecules were involved in the predissociation binding. If we assume that ethidium bromide follows the neighbor exclusion principle, then we conclude that predissociation binding involves ≈17 bp of core particle DNA. Above ν values of 0.30, dissociation was extremely sensitive to further addition of ethidium bromide and seems to asymptotically approach completion at about a ν value of 0.35. Data were not analyzed at higher ratios because of aggregation at the top of the gel (see above).

Characterization of Dissociation. Fig. 3 shows that the ethidium bromide-induced dissociation of the core particle, under low ionic strength conditions, is time-dependent. Quantification of the dissociated DNA band for several ratios

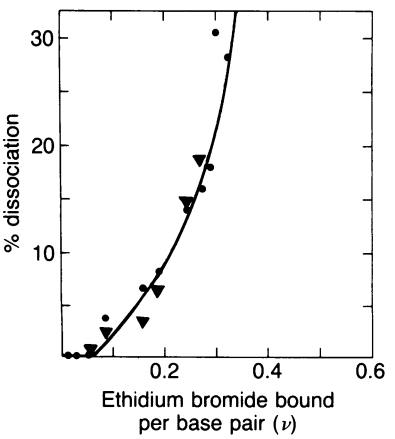

FIG. 6. Plot of percent dissociation versus bound ratio for several core particle–ethidium bromide complexes: (•) data were obtained by method 1, ethidium-stained DNA calibration; (▼) data were obtained by method 2, scanning of ^{32}P-end-labeled core particles. The core particle concentration used for all measurements was 3.8×10^{-5} M bp. The buffer used for all measurements was 10 mM Tris·HCl, pH 8.0/0.1 mM EDTA. The fraction of bound dye was determined for each sample by equilibrium dialysis.

as a function of time revealed that ≈90% of the total dissociation occurred in ≈5 hr. Dissociation was complete in ≈25 hr.

The dissociation of the core particle by ethidium bromide was completely reversible and only dependent on ethidium bromide.

DISCUSSION

We have prepared homogeneous chicken erythrocyte core particles, free from contamination with linker DNA. When ethidium bromide, an intercalating dye, is allowed to form a complex with the folded, nucleosomal DNA, the result is a step-wise dissociation. We have demonstrated this dissociation by both gel electrophoresis and sucrose gradient centrifugation. We have also observed the dissociation in the analytical ultracentrifuge, but these results are more difficult to interpret due to the presence of free dye. Dissociation, at least at low concentrations, is fully reversible and, therefore, represents an equilibrium process.

The dissociation proceeds through at least one intermediate form, a particle containing 145 bp of DNA and a histone hexamer. Since this particle is detected prior to any amount of dissociated DNA, we conclude that the initial loss of one copy each of H2A and H2B is an obligatory step in the dissociation process. If other intermediates are formed, such as one containing a histone tetramer, these structures are not kinetically stable for we have not been able to detect them. Extrapolation of the percent-dissociation versus bound-ratio curve (Fig. 6) to zero dissociation indicated that ≈9 bound ethidium bromide molecules are the critical number required for dissociation to begin. In our estimation of ν_c, we have made the assumption that the fractional population (f) of the core particles that binds the dye is 1.0. If the $f < 1.0$, then the correct value of ν_c is ν_c/f. If we assume that ethidium bromide follows neighbor exclusion binding when bound to the core particle and that $f = 1.0$, then ≈17 bp are involved in the predissociation binding. This value is very close to the size of the putative binding site of an H2A–H2B dimer (23). This value is also close to a theoretically predicted (20 bp) number of sites available for initial binding of ethidium bromide to the native core particle structure (24). The approach of the percent dissociation curve to a limiting bound ratio also suggests that a limited number of sites are available for ethidium bromide binding to the intact structure.

The intercalation binding of ethidium bromide to free DNA has several effects. It unwinds the DNA helix by 26° (25), and induces changes in sugar pucker, glycosidic torsional angle, and phosphodiester torsional angles in DNA and RNA (26–28). These changes in winding angle and conformation produce a slight bending of the DNA helix towards the major groove (28). In general, intercalation of planar molecules causes lengthening and stiffening of the helix (4). If these effects hold for the core particle, it is not surprising that dissociation occurs. We conclude that stiffening of the helix accompanied by changes in the winding angle due to a critical level of ethidium bromide binding prohibit the DNA from bending around the histone octamer. We have observed that the binding of daunorubicin, an intercalator with quite different binding geometry and unwinding angle (12°) (29–31), also induces dissociation of the core particle. Thus, dissociation may be an effect common to the intercalation mechanism. However, the level of dissociation probably results from the sum of winding angle changes, conformational and steric factors tht govern the binding ability of the DNA to the protein core. Therefore, each intercalator probably represents a slightly different case.

We suggest a few experimental consequences of dissociation. First, since dissociation leads to the presence of free DNA, which binds ethidium with a different affinity than do nucleosomes, binding isotherms must be corrected for the levels of free DNA present. To our knowledge, dissociation has never been considered in constructing Scatchard plots for ethidium binding to nucleosome complexes. Second, investigators attempting to measure motional dynamics of the core particle via an intercalating molecule, must exercise caution in describing the type of complex they are measuring. Since partial release of histones is produced, even at low ratios of dye to bp, such studies may reflect only the dynamics of a free DNA–ethidium complex tethered at one end, or even free DNA. Finally, the use of ethidium bromide to relax supercoils and to determine the linking number for nucleoprotein systems will require proof that nucleosome structure is maintained. It should be noted, however, that our results cannot necessarily be extrapolated to the effects of ethidium binding on long chromatin.

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1. Fuller, W. & Waring, M. J. (1964) *Ber. Bunsenges. Phys. Chem.* **68**, 805–808.
2. Lerman, L. S. (1961) *J. Mol. Biol.* **3**, 18–30.
3. Hajduk, S. L. (1978) in *Progress in Molecular and Subcellular Biology*, eds. Hahn, F. E., Kersten, H., Kersten, W. & Szybalski, W. (Springer, Berlin), pp. 158–200.
4. Wilson, W. D. & Jones, R. L. (1981) in *Advances in Pharmacology and Chemotherapy*, ed. Hawking, F. (Academic, New York), Vol. 18, pp. 117–222.
5. Waring, M. J. (1981) *Annu. Rev. Biochem.* **50**, 159–191.
6. Wilson, W. D. & Jones, R. L. (1982) in *Intercalation Chemistry*, eds. Whittingham, M. S. & Jacobsen, A. J. (Academic, New York), pp. 445–501.
7. Waring, J. J. (1972) in *Molecular Basis for Antibiotic Action*, eds. Gale, E. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H. & Waring, M. J. (Wiley, New York), pp. 173–277.
8. Fenske, H., Eichorn, I., Böttger, M. & Lindigkeit, R. (1975) *Nucleic Acids Res.* **2**, 1975–1985.
9. Cartwright, I. L., Hertzberg, R. P., Dervan, P. B. & Elgin, S. C. R. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 3213–3217.
10. Wu, H., Dattagupta, N., Hogan, M. & Crothers, D. M. (1983) *Biochemistry* **22**, 626–634.
11. Erard, M., Das, G. C., de Murcia, G., Mazen, A., Pouyet, J., Champagne, M. & Daune, M. (1979) *Nucleic Acids Res.* **6**, 3231–3253.
12. Wang, J., Hogan, M. & Austin, R. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5896–5900.
13. Hurley, I., Osei-Gyimah, P., Archer, S., Scholes, C. P. & Lerman, L. S. (1982) *Biochemistry* **21**, 4999–5009.
14. Benezra, R., Cantor, C. R. & Axel, R. (1986) *Cell* **44**, 697–704.
15. Sinden, R. R., Carlson, J. O. & Pettijohn, D. E. (1980) *Cell* **21**, 773–783.
16. Pettijohn, D. E. & Pfenningen, O. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1331–1334.
17. Lutter, L. C. (1978) *J. Mol. Biol.* **124**, 391–420.
18. Yager, T. D. & van Holde, K. E. (1984) *J. Biol. Chem.* **259**, 4212–4222.
19. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
20. O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021.
21. Weischet, W. O., Tatchell, K., van Holde, K. E. & Klump, H. (1978) *Nucleic Acids Res.* **5**, 139–160.
22. Tatchell, K. & van Holde, K. E. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3583–3587.
23. Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D. & Klug, A. (1984) *Nature (London)* **311**, 532–537.
24. Schmitz, K. S. (1982) *J. Theor. Biol.* **98**, 29–43.
25. Wang, J. C. (1974) *J. Mol. Biol.* **89**, 783.
26. Tsai, C., Jain, S. C. & Sobell, H. M. (1977) *J. Mol. Biol.* **114**, 301–315.
27. Jain, S. C., Tsai, C. & Sobell, H. M. (1977) *J. Mol. Biol.* **114**, 317–332.
28. Sobell, H. M., Tsai, C., Jain, S. C. & Gilbert, S. G. (1977) *J. Mol. Biol.* **114**, 333–369.
29. Jones, R. L., Lanier, A. C., Keel, R. A. & Wilson, W. D. (1980) *Nucleic Acids Res.* **8**, 1613–1624.
30. Fritzsche, H., Triebel, H., Chaires, J. B., Dattagupta, N. & Crothers, D. M. (1982) *Biochemistry* **21**, 3940–3946.
31. Quigley, G. J., Wang, A. H. J., Ughetto, G., van der Marel, G., van Boom, J. H. & Rich, A. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7204–7208.